

Poster: free communications

Assessment of corneal surface impairment and early signs of eye irritation on reconstructed Human Corneal Epithelium

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Background: ESAC (Ecvam Scientific Advisory Committee) has validated in April 2007 two replacement Alternatives to assess the eye irritation: the Bovine Corneal Opacity Permeability (BCOP) and Isolated Chicken Eye (ICE). The 2 methods will formally replace the Draize test for and will be introduced in the Annex V of Dir. 67/548 /EEC of dangerous substances .

Nowadays the need of pharmaceutical and cosmetic industry beside the regulatory requirements is to discriminate between low, very low or even non irritant products, stressing the concept of eye compatibility more than eye irritation. Two main categories of products represent a real challenge: cosmetics that could strongly differ for ingredient's type and concentration, technical form and mode of application and the long terms ophthalmological treatments (i.e. dry eye) involving repeated application without clinical signs as it is the case of preservatives toxicity .

Material and methods: The HCE model-SkinEthic Laboratories (Nice, F) has been used in this study : Human 3D Tissue cultures of Corneal Epithelium may support basic research investigation and they are versatile for the set-up of modified protocols allowing objective and reproducible quantification of complementary testing parameters.

The Multiple Endpoints Analysis (MEA) Protocol, proposed several years ago, that includes complementary parameters (cell viability, morphology, release of soluble factors as IL-8) has been modified by including the transcriptional regulation of a structural component of the tight junctions as early marker of the effects of sub toxic doses linked to infra-clinical reactions. The mRNA expression has been monitored after different exposures by quantitative RT-PCR with Taqman[®] assay. The modified protocol has been applied to study a category of potentially non toxic products as the commercially available tears substitutes. Toxic and non toxic doses of well known eye irritant as BAK (Benzalkonium Chloride) were used as positive controls.

Results: The results reported in this study have shown that this modified procedure of the MEA approach was able to better discriminate between products that seems to be identical after an acute exposure of 24h. By using the modified protocol that includes a post incubation, a better understanding of the interaction with the corneal surface has been possible. Globally by scoring the different parameters a first discrimination has been done between a strong irritant that is responsible of the degradation of the tissue and a low irritant able to allow tissue recovery or finally to distinguish between different levels of low irritation. The reactivity of corneal epithelium has been also modified with specific conditions of hydration and osmolarity in order to study its reactive defense mechanism.

Discussion: Thanks to its sensitivity the HCE model seems to be promising in predicting the impairment of corneal surface with adapted toxicological protocols.

Keywords: ocular surface, eye irritation, HCE, BAK